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PRINCIPAL INVESTIGATOR: Radoslav Goldman

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Radoslav Goldman
rg26@georgetown.edu

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14. ABSTRACT

In this proposal, we are testing the hypothesis that the risk of developing prostate cancer and the aggressiveness of the disease are influenced by protein glycosylation. We postulate that glycobiology contributes to the higher susceptibility of African American men. The study population is assembled and an annotated sample repository is ready for use. Methods for the glycomic analysis of fractionated proteins are in place. We are evaluating mRNA datasets comparing prostate cancer in African American and Caucasian men. Our aim is to expand the study in order to evaluate the glycomic changes in prostate cancer health disparity.

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INTRODUCTION

In this proposal, we are testing the hypothesis that the risk of developing prostate cancer and the aggressiveness of the disease are influenced by protein glycosylation. We postulate that glycobiology contributes to the higher susceptibility of African American men. A major goal of the study is to evaluate the glycosylation differences in prostate cancer of African American and Caucasian men living in the Baltimore-Washington metropolitan area.

Aim1. Quantify N-glycans in serum of men in a case-control study of prostate cancer with a focus on differences between Caucasians and African Americans.

Aim2. Evaluate prediction accuracy of select N-glycans for the detection of prostate cancer.

Aim3. Perform an exploratory study of N-glycans in urine of the participants and correlation of glycans with gene expression in existing array datasets.

BODY

We have now assembled a study of protein glycosylation which includes comparison of healthy a biopsy controls with cancer cases. We analyze both pooled samples and individual patient samples in a pooled-unpooled study design **(Table 1)**.

The samples are prepared and ready for analysis. These samples will be tested using our newly optimized mass spectrometric methods for quantification of N-glycans. Glycosylation will be quantified by mass spectrometric analysis of permethylated N-glycans enzymatically harvested from serum proteins. We have found that the N-glycosylation of serum proteins is strongly affected by the glycosylation of immunoglobulins **(Figure 1)**. We have therefore included into the work flow a separation of immunoglobulins using protein A and G microcolumns. This will allow us to analyze separately the N-glycans associated with immunoglobulins (immune system related glycosylation) and remaining proteins (non-immune glycosylation response). We plan to carry out these analyses on the samples described in Table 1 in the second year of our study.

As a first study, we have completed the analysis of the enzymatically detached N-glycans in repeatedly sampled samples of 20 healthy controls (50% African American). For each of the participants, we have obtained four blood samples in the span of six months. We have thus generated mass spectra on 80 samples (4x20 samples) while separating immunoglobulins from the remaining proteins. This study will allow us to evaluate the variability of the protein N-glycosylation in apparently disease free general population. We have already completed the analyses and are currently summarizing the results. We hope to have a summary of the results ready for publication within the next 2 months.

In addition, we have begun a comparative analysis of gene expression in African American (AA) and European American (EA) men in order to identify differentially expressed glycogenes. Glycogenes were defined as probes selected by the Consortium for Functional Glycomics (CFG) for inclusion on their mRNA array (http://www.functionalglycomics.org/static/consortium/resources/resourcecoree.shtml). The array contains probe sets for 1175 unique glycogenes. We looked for intersection of this gene set with the genes observed as differentially abundant in GSE6956 (Wallace et al, 2008, PMID: 18245496) and GSE17356 (Timofeeva et al, 2009, PMID: 19724911). These are the only two mRNA expression studies we identified comparing the expression in prostate cancer of AA and EA men. GSE6956 contains data of 89 samples; prostate tumor (n=69) and non-tumor tissue (n=20). We used the array data of 69 tumor samples for our study. Samples in GSE17356 are primary prostate cancer epithelial cell cultures (n=27). The authors compared the mRNA expression in prostate cancer samples isolated from AA and EA men.

By using the SAMR package and Bayesian regularized t test on re-annotation profile, we identified 28 glyco genes among the differentially expressed mRNA in GSE6956 and 40 glyco genes among the differentially expressed mRNA in GSE6956. We will further evaluate whether the glycosylation related genes identified in our analysis affect prostate cancer disparity and glycan profiles observed in our study. Comparison of mRNA expression in GSE6956 and GSE17356 showed 40 genes consistently up- or down- regulated in both sets (comparison of the AA and EA men). The genes are listed in **Table 2**. Two of the genes belong to the glycogene set **(Table 3)**. A strong association was found between the dysregulated genes and the insulin regulation of fatty acid metabolism.

KEY RESEARCH ACCOMPLISHMENTS

1. The study population is assembled and an annotated sample repository is ready for use.
2. Methods for the glycomic analysis of fractionated proteins are established
3. Separation of immunoglobulin associated glycans improves glycomic analyses
4. mRNA array informatics show 40 gennes differentially abundant consistently in two existing datasets but these are the opnly two datasets comparing African American and Caucasian men we identified

REPORTABLE OUTCOMES

NA

CONCLUSION

The study proceeds along the projected aims. We have extablished all the methods and plan to analyze a sufficient number of samples to evaluate the glycomic changes in prostate cancer health disparity.

REFERENCES

NA

Appendices

NA

Supporting Materials

Source	Cases		Healthy controls		Biopsy controls	
Race	CA	AA	CA	AA	CA	AA
Sample number	70	70	50	50	38	38
Pool number	7	7	5	5	4	4

Table 1. Study design includes Caucasian American (CA) and African American (AA) men in each category of cancer cases, healthy population controls, and biopsy controls verified to be cancer free. Pools represent groups of 10 or 8 patient samples pooled for an exploratory analysis.

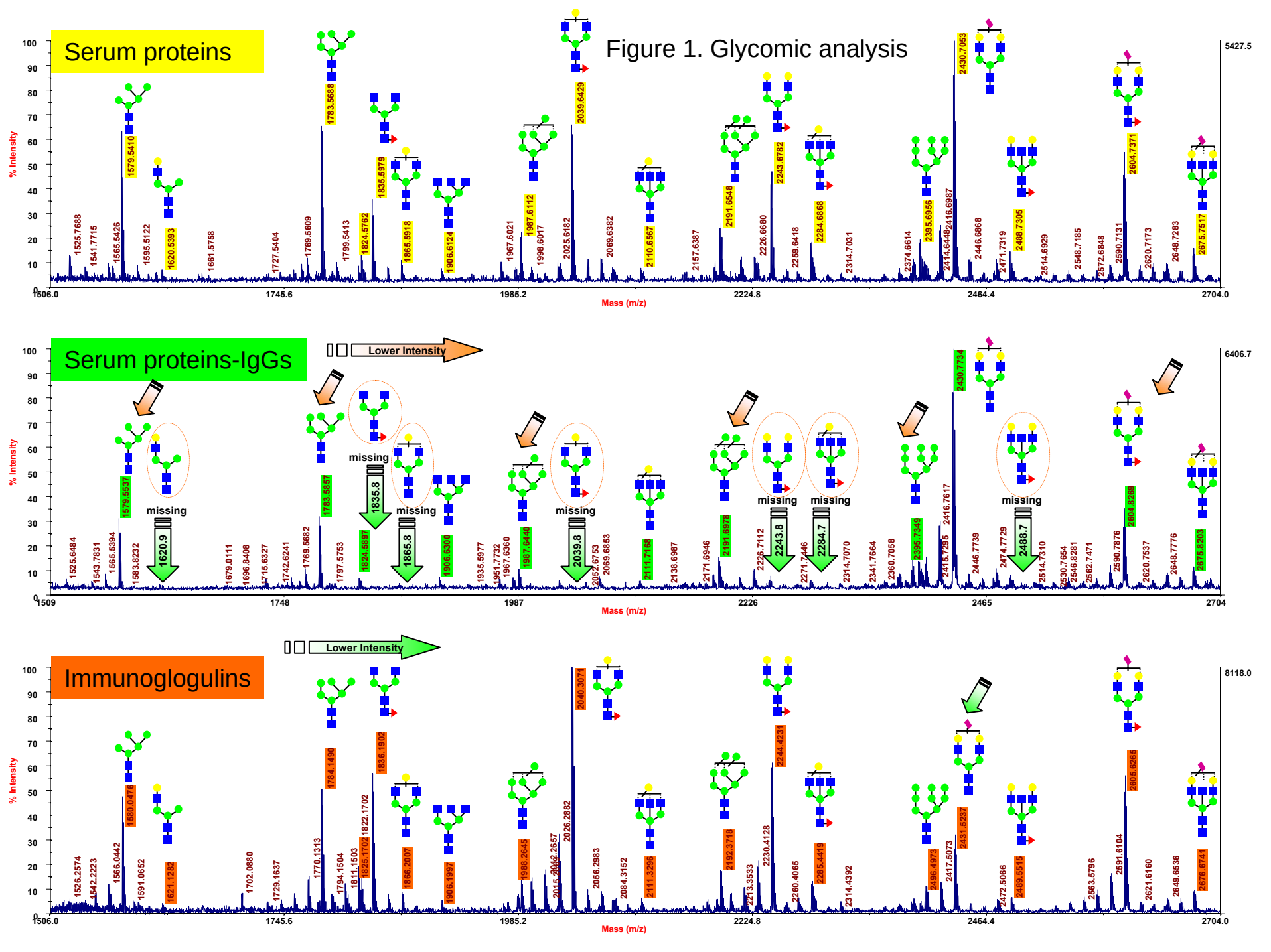
Gene symbol	FC_6956	p_6956	FC_17356	p_17356
ADI1	0.65	3.04E-05	0.46	3.04E-05
AMFR	1.68	4.29E-05	3.00	3.01E-07
APIP	0.83	5.49E-03	0.66	3.94E-03
ATP11B	0.83	6.23E-03	0.74	5.34E-03
BIN2	1.17	5.62E-03	1.16	9.55E-03
C14orf108	0.84	3.56E-04	0.72	7.39E-04
C18orf10	1.16	8.56E-04	1.20	7.54E-03
C3orf37	0.90	3.83E-03	0.75	6.45E-04
C7orf49	0.89	2.60E-03	0.80	2.63E-03
CLC	0.91	2.34E-04	0.89	6.06E-03
CNNM4	0.91	8.74E-03	0.80	1.55E-03
CPSF4	0.92	6.05E-03	0.86	5.04E-03
CRYBB2	1.93	7.80E-11	2.20	1.34E-04
CTNNB1	1.37	7.18E-07	2.13	3.91E-07
EBI2	1.55	4.06E-03	1.30	5.57E-03
FAM128A	0.76	1.36E-03	0.63	9.08E-03
FASTKD3	0.86	8.46E-03	0.70	2.99E-03
GOLPH4	1.18	1.33E-03	2.05	7.47E-10
IL20RA	0.71	2.15E-03	0.70	6.00E-04
INDO	1.39	2.17E-03	1.26	5.65E-04
LEPROT	1.19	6.06E-03	1.24	2.59E-03
MAP3K15	1.20	9.75E-06	1.28	8.42E-03
MAPK8	0.89	4.02E-03	1.16	8.05E-04
MGAT1	1.07	8.32E-03	1.19	9.84E-03
MRPL35	0.89	6.66E-03	0.81	8.53E-03
MRPS7	0.89	5.97E-03	0.80	1.70E-03
MTA1	0.89	5.05E-03	1.22	6.64E-03
MXRA7	1.38	7.59E-04	1.35	8.08E-03
NARS2	0.78	2.03E-04	0.75	3.12E-04
PAPD1	0.88	1.29E-04	0.81	4.44E-03
PRPSAP1	0.88	9.89E-04	0.80	4.45E-03
PSPH	2.34	1.70E-09	2.02	9.52E-03
RFX5	1.10	1.53E-03	0.79	3.30E-03
RPP38	0.87	6.80E-04	0.71	8.95E-06
SFXN1	0.88	2.89E-03	0.56	3.49E-03
SOS1	1.27	1.53E-03	1.67	9.72E-07
TTC27	0.91	9.38E-03	0.74	2.70E-03
VPS53	0.92	4.92E-03	1.24	1.06E-03
VRK2	0.88	2.87E-03	0.80	1.50E-03
ZNF227	0.87	1.87E-03	0.81	2.97E-03

Table 2. mRNA expression of 40 genes consistently up- or down- regulated in the comparison of the AA and EA men in both GSE6956 and GSE17356 datasets.

Probe	Gene Symbol	FC_6956	p_6956	FC_17356	p_17356
202377_at	LEPROT	1.19	6.06E-03	1.23	2.59E-03
201126_s_at	MGAT1	1.06	8.32E-03	1.19	9.84E-03

Gene Symbol	Source1	Source2	Name	NM
LEPROT	xGrowth Factors & Receptors	Miscellaneous	LEPROT [leptin receptor gene-related protein]	NM_017526
MGAT1	Glycan-transferase	GlcNAc-T	MGAT1 (mannosyl (alpha-1,3-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase)	NM_002406

Table 3. Glycogenes selected from Table 2.


Serum proteins
Figure 1. Glycomic analysis
% Intensity
Mass (m/z)
Serum proteins-IgGs
Lower Intensity
missing
Immunoglogulins
Lower Intensity